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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/19, A61K 38/19, 38/48, C07K 16/24, G01N 33/68, C07K 14/52, C12N 5/10

A2

(11) International Publication Number: WO 99/28474

(43) International Publication Date: 10 June 1999 (10.06.99)

(21) International Application Number: PCT/US98/25492

(22) International Filing Date: 1 December 1998 (01.12.98)

(30) Priority Data:
60/067,033 1 December 1997 (01.12.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/067,033 (CON)
Filed on 1 December 1997 (01.12.97)

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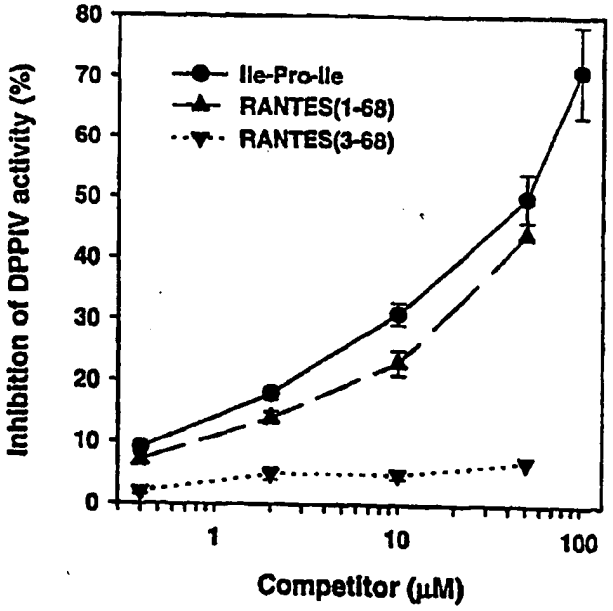
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: CHEMOKINE VARIANTS AND METHODS OF USE

(57) Abstract

The present invention provides the nucleotide and amino acid sequence of truncated RANTES (3–68), which has the same amino acid sequence as the wild–type RANTES, but with a Serine/Proline truncation at positions 1 and 2 from the N–terminus, respectively. CD26 is a leukocyte activation marker that possesses dipeptidyl peptidase IV (DPPIV) activity but whose natural substrates and immunological functions had not been previously defined. Several chemokines, including RANTES (regulated on activation, normal T expressed and secreted) are provided, which are substrates for human CD26. The truncated RANTES (3–68) lacked the ability of native RANTES (1–68) to increase the cytosolic calcium concentration in human monocytes, but it still induces this response in macrophages activated with macrophage colony–stimulating factor (M–CSF). RANTES (3–68) retains the ability of stimulate CCR5 receptors and to inhibit the cytopathic effects of HIV–1. The invention provides methods for identifying compounds that affect DPPIV–mediated chemokine cleavage, methods for inhibiting HIV infection and treating individuals having or at risk of having HIV infection, methods for diagnosis and/or prognosis of individuals having a chemokine–associated disorder and methods for accelerating wound healing and angiogenesis, all based on the discovery of DPPIV–mediated cleavage of chemokines.



dipeptidyl peptidase IV (DPPIV), thereby truncating the chemokine and producing a variant chemokine. Chemokines may include, but are not limited to, RANTES, MIP-1, IP-10, eotaxin, MDC, and MCP-2.

The invention also provides a method for inhibiting HIV-1 replication in a host cell susceptible to HIV-1 infection, comprising contacting the cell or the host with an effective amount of dipeptidyl peptidase IV (DPPIV) enzyme such that macrophage-derived chemokine (MDC) is cleaved to produce truncated MDC, thereby providing antiviral activity and inhibiting HIV-1 replication and a A method for inhibiting HIV-1 replication in a host cell susceptible to HIV-1 infection, comprising contacting the cell or the host with an effective amount of dipeptidyl peptidase IV (DPPIV) enzyme such that RANTES is cleaved to produce truncated RANTES, thereby providing antiviral activity and inhibiting HIV-1 replication.

In another embodiment, the invention provides a method for inhibiting dipeptidyl peptidase IV (DPPIV)-mediated chemokine processing comprising contacting DPPIV with an inhibiting effective amount of a compound which inhibits DPPIV expression or activity.

In another embodiment, the invention provides a method for inhibiting an allergic or inflammatory reaction in a subject, comprising administering to the subject an effective amount of Dipeptidyl peptidase IV (DPPIV) enzyme such that a chemokine is cleaved to produce a truncated chemokine, thereby inhibiting an allergic or inflammatory reaction. Preferably, the chemokine is eotaxin.

In another embodiment, the invention provides a method for accelerating angiogenesis or wound healing in a subject, comprising administering to the subject an effective amount of an inhibitor of dipeptidyl peptidase IV (DPPIV) enzyme activity or gene expression or a DPPIV-insensitive chemokine, such that chemokine processing is inhibited, thereby accelerating angiogenesis or wound healing. One exemplary chemokine useful in the method for accelerating angiogenesis is IP-10.

In all of the above methods, the exemplary DPPIV shown in the present invention is CD26.

In yet another embodiment, the invention provides a method for diagnosis or prognosis of a subject having a chemokine-associated disorder. The method includes

Preferably, a chemokine useful for inhibition allergic or inflammatory reactions is a truncated eotaxin.

The use of a truncated chemokine in the method of the invention may inhibit or depress an immune or inflammatory response where desirable, such as in graft rejection responses after organ and tissue transplantations, or autoimmune disease. Some of the commonly performed transplantation surgery today includes organs and tissues such as kidneys, hearts, livers, skin, pancreatic islets and bone marrow. However, in situations where the donors and recipients are not genetically identical, graft rejections can still occur. Autoimmune disorders refer to a group of diseases that are caused by reactions of the immune system to self antigens leading to tissue destruction. These responses may be mediated by antibodies, auto-reactive T cells or both. Some important autoimmune diseases include diabetes, autoimmune thyroiditis, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosis, and myasthenia gravis. Other allergic or inflammatory responses are included in the method of the invention.

In another embodiment, the invention provides a method for accelerating angiogenesis or wound healing in a subject, comprising administering to the subject an effective amount of an inhibitor of dipeptidyl peptidase IV (DPPIV) enzyme activity or gene expression or a DPPIV-insensitive chemokine, such that chemokine processing is inhibited, thereby accelerating angiogenesis or wound healing. For example, new blood vessels are required for tissue repair and enhanced blood vessel growth may aid in improving circulation to ischemic limbs and heart tissue suffering from atherosclerotic disease, healing skin ulcers or other wounds, and establishing tissue grafts. Preferably, a chemokine useful for accelerating angiogenesis is a wild-type IP-10. Cleavage of IP-10 appears to inactivate the activity of IP-10, therefore it is desirable to inhibit cleavage of IP-10. Alternatively, it may be desirable to provide a variant IP-10 polypeptide which contains an amino acid substitution at position 2, such that neither proline nor alanine is present, which would result in a DPPIV-insensitive chemokine. However, such a variant must retain the activity of wild-type IP-10, *e.g.*, a chemoattractant for NK cells.

15. The method of claim 14, wherein the contacting is by *in vivo* administration to a subject.

16. The method of claim 14, wherein the polypeptide is administered by intravenous, intramuscular or subcutaneous injection.

17. The method of claim 14, wherein the polypeptide is formulated in a pharmaceutically acceptable carrier.

18. A method of treating a subject having or at risk of having an HIV infection or disorder, comprising administering to the subject, a therapeutically effective amount of a polypeptide of SEQ ID NO:2, wherein the polypeptide inhibits cell-cell fusion in cells infected with HIV.

19. The method of claim 18, wherein the subject is suffering from AIDS or ARC.

20. The method of claim 18, wherein the polypeptide is formulated in a pharmaceutically acceptable carrier.

21. A method of treating a subject having an HIV-related disorder associated with expression of CCR5 comprising administering to an HIV infected or susceptible cell of the subject, a polypeptide of SEQ ID NO:2 or a nucleic acid sequence encoding the polypeptide of SEQ ID NO:2 or other variant chemokine.

22. The method of claim 21, wherein the polypeptide or nucleic acid is introduced into the cell using a carrier.

23. The method of claim 22, wherein the carrier is a vector.

24. The method of claim 21, wherein the administering is *ex vivo*.